C/EBPβ isoforms and the regulation of metabolism

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Chapter I

Aim and outline of the thesis

Aim of thesis

The transcription factor C/EBPβ is known to regulate metabolism, cell proliferation and differentiation. Its transactivation activity is largely determined by the ratio between the long isoform C/EBPβ-LAP and short isoform C/EBPβ-LIP. Therefore, it is essential to investigate the functions of the single isoforms to better understand the role of C/EBPβ in the cell. The aim of this thesis was to investigate upstream regulators and downstream effects of C/EBPβ isoforms in physiology and pathophysiology (cancer). The first part of the thesis answers the questions whether mTORC1 signalling stimulates C/EBPβ-LIP expression *in vivo* and (if yes) whether LIP is an important downstream mediator of physiological effects of mTORC1. We therefore investigated whether deficient C/EBPβ-LIP expression in mice has a similar effect on animal physiology and metabolism as was observed in other animal models with reduced mTORC1 signalling. The second objective of this thesis was to investigate effects of the single C/EBPβ isoforms LAP and LIP on cellular metabolism. Furthermore, by performing transcriptome and proteome analysis of cells expressing either LAP or LIP we aimed to identify downstream mediators of the different C/EBPβ isoforms in the regulation of cellular metabolism. As a third objective, we addressed whether the changes in cell metabolism caused by the different C/EBPβ isoforms impose metabolic vulnerability onto cancer cells that could be exploited for novel cancer therapy options by using C/EBPβ as a biomarker.

Outline of the thesis

Chapter II reviews the metabolism of proliferating and cancer cells and the function of selected oncoproteins and tumour suppressors in the regulation of cellular metabolism. We describe how glucose, glutamine and mitochondrial metabolism contribute to cell growth and proliferation by supporting *de novo* biosynthesis processes that are altered in cancer cells. In addition, we describe regulators and regulatory mechanisms that control cell and cancer metabolism.

In chapter III, we describe the role of mTORC1-4E-BP signalling in the control of C/EBPβ-LIP expression. We found that high mTORC1 activity stimulates LIP translation *in vivo*. Additional *in vitro* analysis revealed 4E-BPs as critical mTORC1 downstream targets in the regulation of LIP expression. Furthermore, we used a mouse model with deficiency in LIP translation (C/EBPβ$^{\Delta uORF}$ mice) to investigate whether low LIP expression has similar effects on animal metabolism as found in other mouse models with reduced mTORC1 signalling. Mouse models with reduced mTORC1 signalling (e.g. 4E-BP1 overexpressing mice, S6 kinase1 knock-out mice, mTORC1 inhibition by rapamycin or calorie restriction) show a general healthy metabolism and a delay in age-associated phenotypes. We find that LIP deficient C/EBPβ$^{\Delta uORF}$ mice show a lowered body weight, less steatosis and increased glucose and insulin tolerance and thereby resemble the phenotype of other mouse models with decreased mTORC1 signalling or calorie restriction.

In chapter IV, we study the function of C/EBPβ isoforms in cellular metabolism and analyse the effects of the single isoforms on transcriptome and proteome of the cell. We describe that LIP induces energy production and metabolic reprogramming of the cell. The analysis of the transcriptome and proteome points towards a post-transcriptional mechanism induced by LIP to control cellular metabolism. Further analysis of the omics data and CRISPR/Cas9-based knockout identified LIN28B as important downstream mediator of metabolic functions of LIP. Furthermore, we provide evidence that all members of the LIN28B-regulating microRNA family let-7 are novel C/EBPβ targets that are transcriptionally downregulated by LIP resulting in the de-repression of LIN28B. Further, we present a new mouse model (R26LIP) that uses the endogenous Rosa26 locus to introduce a transgenic LIP expression cassette resulting in the ubiquitous overexpression of LIP in all investigated tissues. LIP

overexpressing R26LIP mice show a decreased let-7 and increased LIN28B expression in skin and bone marrow cells, which supports the results of our *in vitro* analysis. Furthermore, these mice develop a hyperplasia in skin and show a metabolic reprogramming in bone marrow cells thereby resembling the phenotypes of LIN28 overexpressing mice.

In chapter V, we investigate whether LIP induced metabolic changes addict cells to specific nutrients and metabolic pathways. We see that cells with high LIP expression are sensitive to glucose starvation and inhibition of glycolysis (2-deoxyglucose). In addition, we show that LIP reprograms cytoplasmic NADH utilisation by increasing the malate-aspartate-shuttle and usage of electrons from cytoplasmic NADH for mitochondrial ATP production. We show that the increased NADH oxidising capacity of the induced malate-aspartate-shuttle causes the 2-deoxyglucose mediated cell death in cells with high LIP expression. Inhibition of NADH oxidising cellular processes (malate-aspartate-shuttle and lactate dehydrogenase) reduces the sensitivity of cells to 2-deoxyglucose while a treatment with an NADH oxidizing drug, duroquinone, increases the sensitivity to 2-deoxyglucose in resident cells. Therefore we hypothesize that the NADH/NAD+ ratio is critically involved in mediating 2-deoxyglucose caused cell death in cells with high LIP expression. This survival dependency of cells with high LIP expression on glycolysis was not only shown in cells ectopically expressing LIP but also in human triple negative breast cancer cell lines that have high endogenous LIP levels and therefore could have an implication for cancer biology.

In Chapter VI, I discuss the studies presented in this thesis in a broader context and I suggest further experiments. In addition, I discuss a possible role of LIP in cell migration and metastasis, and in the regulation of protein translation. Furthermore, I suggest a nutrient level controlled negative feedback loop for LIP expression and a positive signalling feedback loop that involves mTORC1, LIP and let-7.